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# FIRST OBSERVATION OF PARASITIC RELATIONS BETWEEN BIG-HEADED FLIES, *NEPHROCERUS* ZETTERSTEDT (DIPTERA: PIPUNCULIDAE) AND CRANE FLIES, *TIPULA* LINNAEUS (DIPTERA: TIPULIDAE: TIPULINAE), WITH LARVAL AND PUPARIAL DESCRIPTIONS FOR THE GENUS *NEPHROCERUS*

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*Abstract.*—*Nephrocerus atrapilus* Skevington 2005 (Diptera: Pipunculidae) and *Nephrocerus daeckei* Johnson 1903 were reared as endoparasitoids of three species of adult crane flies in the genus *Tipula* Linnaeus (Diptera: Tipulidae). Two additional *Tipula* species were observed to host pipunculid larvae presumed to be species of *Nephrocerus*. Pipunculid larvae are known to parasitize auchenorrhynchous Hemiptera, particularly Cicadellidae, Delphacidae and Cercopidae but this is the first report of hosts for *Nephrocerus*, and the first recorded instance of adult Tipulidae being parasitized by another true fly. The rate of pipunculid parasitism of female crane flies in this study from all collecting sites was 42% (82/193), but the rate for males was 0.008% (1/119). Endoparasitoid pipunculid larvae undergo a rapid active feeding stage for less than 20 days, and then enter an intensive diapause for ten months before pupariation. External anatomical features of larvae and puparia of *Nephrocerus* are described and illustrated. Techniques for collecting and rearing *Nephrocerus* are described.

*Key Words:* Diptera, Pipunculidae, Tipulidae, *Nephrocerus*, larva, puparium, *Tipula*, parasitoids, host records

Big-headed flies, family Pipunculidae, are worldwide in distribution, with 155 species and five subspecies recorded from the Nearctic Region (De Meyer 1996, De Meyer and Skevington 2000). The genus *Nephrocerus* is recently revised and has 18 extant species occurring mainly in the Holarctic Region (six of these in the Nearctic Region) (Skevington 2005). Pipunculid larvae are known to be parasitoids of auchenorrhynchous hemipterans. Skevington and Marshall (1997) presented a definitive summary of known Nearctic pipunculid host associations and indicated pipunculids as being exclusive endoparasitoids of various families of leafhoppers and planthoppers. The list of host families known to be parasitized includes Cercopidae, Cicadellidae, Cixiidae, Delphacidae, Flatidae, Fulgoridae, and Membracidae (Skevington and Marshall 1998). These authors also predicted the likelihood that *Nephrocerus* species would have life histories different from those of other big-headed flies, based on their larger size and different appearance from other pipunculids.

Species of Tipulidae s.l., or crane flies, make up the largest family of the order Diptera or true flies. In North America, more than 1,500 species of crane flies have been described, and 581 species belong to the subfamily Tipulinae (Tipulidae s.s. Oosterbroek 2005). Most species of Tipulinae are of larger body size than the remaining species of Tipulidae. The genus *Tipula* contains about 495 Nearctic species (Oosterbroek 2005) in 27 subgenera (Alexander 1965). The known parasitoids of crane flies include tachinid flies that attack the larval stages (Rennie and Sutherland 1920; Arnaud 1978). Arnaud (1978) lists two genera and five species of tipulid hosts being utilized by six species of tachinids in five genera. Gelhaus (1987) noted *Allophorocera arator* (Aldrich) (Diptera: Tachinidae) as a parasitoid of the larva of *Tipula* (*Triplicitipula*) sp., probably *flavoumbrosa* Alexander (Diptera: Tipulidae). At least two species of *Nephrocerus* are here documented to be parasitoids of adult crane flies in the genus *Tipula*.

Field collecting of crane flies for a faunistic survey of Pennsylvania's Tipulidae (Young and Gelhaus 2000) unexpectedly revealed an unknown parasitic relationship between two true flies. During field collecting, adult crane flies were routinely captured with an aerial net and dispatched in a translucent killing bottle containing potassium cyanide. In 1997 the senior author (DPK) observed a small maggot erupting from the abdomen of an adult crane fly, a male of *Tipula (Lunatipula) submaculata* Loew. As this host fly became anesthetized inside the killing bottle and then completely motionless, a yellow larva penetrated the left pleural membrane of the second abdominal segment, extricated itself completely from the host in a few seconds, and began to crawl about in the bottle. The larva was preserved directly in 80% aqueous ethanol. The identity of this larva could not be determined, but was presumed to belong to the parasitic fly family Tachinidae.

Further fieldwork (2000–2004) at other study sites resulted in observation of additional, similar instances of parasitization. Several more larvae were observed erupting from adult crane flies, thus prompting the authors to keep the larvae alive in order to rear adult parasitoids that would permit species-level determination. Thirty-one larval parasitoids were successfully reared and emerged as adult pipunculid flies. These were determined to be species of *Nephrocerus* Zetterstedt using the keys of Hardy (1943, 1987). This was an exciting and unexpected discovery as they were originally expected to be tachinid flies. Jeffrey H. Skevington later identified 24 flies as *Nephrocerus atrapilus* Skevington, and two as *Nephrocerus daeckei* Johnson. The successful rearing of larvae to adults allows the present study to contribute the first record of host-parasitoid relationships of big-headed flies and crane flies, as well as descriptions and illustrations of larvae and puparia for the genus *Nephrocerus*.

## Materials and Methods

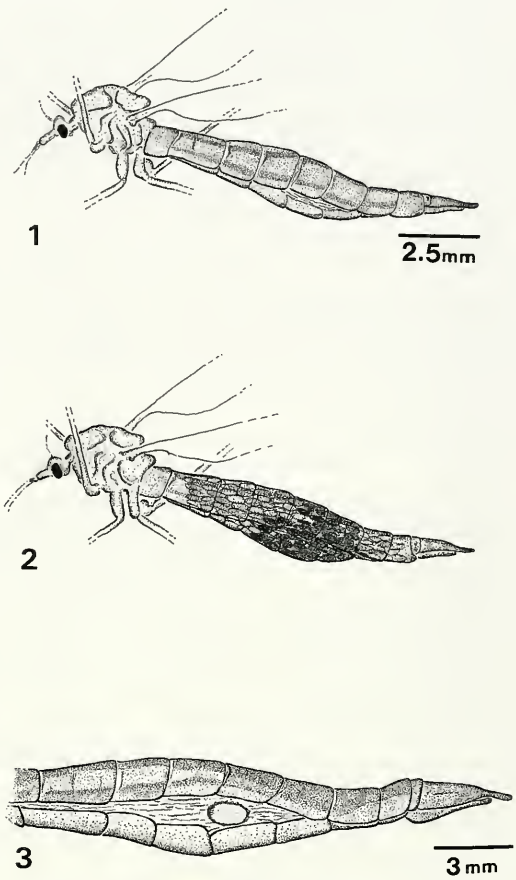
Field observations and collections.—The primary collecting site for this study is located at Boyce Regional Park (40°-27′-42″N, 79°-45′-26″W; elevation 375 m), a suburban park east of Pittsburgh, Allegheny County, Pennsylvania. The habitat is a moderately mesic, second growth, mixed-deciduous forest. Boyce Park is in the Pittsburgh Low Plateau Ecological Region with the rolling hills typical of the topography of that region. There are utility rights-of-way and foot trails creating a mosaic of forest plots, forest edges, and open canopy spaces with uncut grass and wildflowers. The hardwood forest is diverse, dominated by *Prunus serotina* Ehrh. (black cherry; Rosaceae), species of *Acer* (maples; Aceraceae) and *Sassafras albidum*

(Nutt.) Nees (sassafras; Lauraceae). Common shrubs are *Lindera benzoin* (L.) Blume (spicebush; Lauraceae), *Rosa multiflora* Thunberg ex Murr. (multiflora rose; Rosaceae) and species of *Rubus* (blackberry; Rosaceae). Forest undergrowth includes vines, herbaceous plants, and grasses. Forest leaf litter and leaf molds are present over most of the level area and gentler slopes. The larvae of many terrestrial *Tipula* species feed on the detritus and mold just beneath the leaf litter. Both woodland and grassland species of crane flies are common in this habitat.

A total of twenty-six diurnal collecting forays was conducted over the course of five collecting seasons from June through August between 2000 and 2004. Aerial net sweeping was the basic method of collecting. Adult crane flies were taken either at resting sites among the vegetation, or in the air as they flew. Crane flies of both sexes were collected and kept individually to ensure positive host-parasitoid association with any emerging parasitic larvae. Holding jars for captured flies were plastic (5 cm diameter × 6.5 cm tall) with screw-top lids.

Some crane flies were recognized as being parasitized by a distinct swelling restricted to the middle abdominal segments (Fig. 1). Specimens with this condition would often, but not always, host a parasitic larva. Superficially, these infected female flies appeared gravid (Fig. 2), except no black eggs were visible; black, mature eggs are visible through many abdominal segments of truly gravid females. On occasion, a parasitic larva would unexpectedly emerge during specimen retrieval from the aerial net or when the host fly was placed in a killing jar. Most larvae emerged later while host flies were in their separate holding jars.

Early collections at the primary site produced only female hosts. This observation resulted in the later forays with a collection bias toward female specimens in an effort to obtain more pipunculid larvae. Adult crane flies of larger species can be sexed visually in the field from a distance of about two meters as they rest on top of vegetation. The outline of the caudal abdominal segments is expanded and round in males. In contrast, the female has a tapering abdomen that terminates with an acute ovipositor. In addition, crane flies can be sexed by their flight pattern in the air. Males have an erratic flight with undulations and spiral rotations along the axis of travel. Females maintain a more direct, steady, and straight flight path.




Figs. 1–3. Adult female of *Tipula (Lunatipula) duplex*. 1, Parasitized female, left lateral view. 2, Non-parasitized gravid female, left lateral view. 3, Exit hole of *Nephrocerus* larva on parasitized female, left lateral view.

Rearing methods and observations.—Adult crane flies were collected and held in separate plastic jars at approximately room temperature (19–26°C). A few drops of tap water were placed in each holding jar and often consumed by the crane flies. The flies were kept alive to allow the pipunculid larvae, if present, to reach maturity before they left the host. The flies were checked daily to search for recently emerged pipunculid larvae. As emerged larvae were first spotted, the condition of the host was recorded as being either alive or dead. Dead host specimens were submerged in Peterson's fixative (1:1:2:9 kerosene, dioxane, acetic acid, 95%ethanol) for twenty-four hours, and then transferred to 70% ethanol to await dissection.

Prepupae were transferred into separate round plastic culture dishes (8 cm diameter × 3 cm tall) with airtight, snap lids. These culture dishes were filled with soil from the collecting sites mixed with commercial potting soil to a depth of approximately 1.5 cm. The medium was kept moist at all times. Newly introduced larvae immediately dug down into the medium. Cultures were inspected multiple times over the next ten months with the aid of a dissecting microscope. Inspections helped to monitor larval health, movement, and pupariation. Recently expired individuals were salvaged for morphological study.

Observation showed that prepupae may crawl through the medium for a period of time during the summer and fall months, remain quiescent for a long period, and may resume movement again prior to pupariation the following spring. Prepupae were never seen exposed on top of the soil medium. Prepupae usually became darker in color, changing from pale yellow or white to honey-brown, in the days following their initial introduction into the soil. They would remain this color until pupariation. As prepupae became less active they also appeared more contracted and wrinkled.

The prepupae were held at temperatures that roughly coincided with the outside seasonal temperatures. The range throughout the months of June to August was 19–26°C, for September through November, 13–21°C. All specimens were placed in a refrigerator at approximately 2°C for about 98 days, from December through February. The cultures were removed from the refrigerator and stored at 12–14°C in March and part of April, and at 15–25°C through April and May when outside temperatures steadily rose. Light levels and photoperiod were not monitored or controlled in any manner.

Puparia always develop in the spring. The overwintering prepupae would "awaken" after a long period of inactivity and start moving through the soil. After a short period of movement, the prepupae settled into another quiescent period prior to pupariation. Adult pipunculid flies would eclose, sometimes dragging the pupal exuviae to the surface of the soil. No attempt was made to maintain or breed the adult pipunculids in captivity and their potential adult longevity was not studied. The intact adult specimens were frozen and later pinned or pointed. Specimens that did not eclose successfully or failed to harden their teneral cuticle properly were preserved in 70% ethanol.

Dissection of *Tipula* host.—Approximate body length was recorded from the tip of the nasus to the tip of the abdomen. All measurements are approximate due to curvature of the abdomen and the head being deflected downward. Under the dissecting microscope, the abdomen was cut and separated at the base of the first segment. The inner surface of the metathorax was inspected for any possible damage by the parasitoid. The abdomen was cut along its full length through the middle of the ster-

Table 1. Parasitization of adult female *Tipula* by *Nephrocerus* larvae, 2000–2004, at Boyce Regional Park, Allegheny County, Pennsylvania.

| *Tipula* spp. (Females Only) | No. of *Tipula* Observed | No. of *Tipula* Infested | % of Hosts Infested | No. of Parasitoid Larvae Emerged | No. of Parasitoid Larvae Found *in situ* by Dissection | No. of *Nephrocerus atrapilus* Reared | No. of *Nephrocerus daeckei* Reared |
|---|---|---|---|---|---|---|---|
| *T.(L.) duplex* | 156 | 69 | 44 | 49 | 21 | 15 ♂ 13 ♀ | 0 |
| *T.(L.) mallochi* | 24 | 9 | 38 | 6 | 3 | 0 | 1 ♂ 1 ♀ |
| *T.(L.) submaculata* | 9 | 2 | 22 | 1 | 1 | 0 | 1 ♂ |
| *T.(B.) borealis* | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| TOTALS | 191 | 80 * | 42 | 56 * | 25 * | 28 | 3 |

* The discrepancy between the sum of total *Nephroceus* parasitoids (81) and the total *Tipula* infested (80) is due to the fact that one dissected female contained two parasitoid larvae.

nites. The pleural membranes were kept intact as many larvae were observed to exit through that lateral part of the abdomen. Exit holes of some larvae were located (Fig. 3), their positions recorded, and the contents of the abdomen were noted. Specimens thought to be unparasitized were dissected and their contents noted for comparison. Some of these without distinctive abdominal swelling contained pipunculid larvae. The location and orientation of pipunculid larvae within the host's abdomen was recorded. Dissected specimens were returned to 70% ethanol for permanent storage.

## Results

Results from collection and rearing.—Over five collecting seasons, 2000–2004, a total of 309 specimens of *Tipula* (118 males and 191 females) was captured at Boyce Park and observed in captivity for parasitoid infestation. Eighty specimens (26% of total crane flies examined) were found to be infested with an endoparasitic larva and all of these were female (therefore 42% of captured female hosts were infested). In 2003, the most productive season, 159 specimens of *Tipula* (33 males and 126 females) were captured for study; of these, sixty females were infested (38% of total crane flies observed, and 48% of the females).

Eight species of *Tipula* were collected at the primary study site. The two most common species, *Tipula (Lunatipula) duplex* Walker and *Tipula (Lunatipula) mallochi* Alexander are recorded here as hosts for larvae of *N. atrapilus* Skevington. A third species, *T. (L.) submaculata* Loew, as well as *T. mallochi*, are here documented as hosts of larvae of *Nephrocerus daeckei* Johnson (see Table 1). All infested hosts at this site were females with body lengths of 20 to 28 mm. The remaining species observed in captivity were: *Tipula* (*Beringotipula*) *borealis* Walker, *T.* (*L.*) *johnsoniana* Alexander, *T.* (*L.*) *flavibasis* Alexander, *T.* (*Triplicitipula*) *colei* Alexander, and *T.* (*Yamatotipula*) *tricolor* Fabricius. No male crane flies were found to harbor parasitic larvae in this site. Except in one instance, infested female crane flies contained only one parasitic pipunculid larva each.

Female *Tipula* flies lived from 1–8 days in captivity. Males lived from 1–11 days. The age of these flies at the time of collection is unknown. A teneral female, collected as it eclosed from the pupal exuvium on the forest floor, lived for eight days in captivity. The time elapsed from host fly capture to parasitoid larval emergence was 1–5 days. The condition of the host crane fly was recorded at the time of larval emergence. Many host flies were still alive after

parasitoid emergence, most for approximately one additional day. Others lived less time and were found lying on their sides motionless or only twitching. A number of hosts were found dead with an associated, emerged parasitoid larva.

Fifty-six cultures of parasitoid larvae were maintained after emergence from their hosts, overwintered under refrigeration, and some were reared to pupariation and adults in the spring. A total of 31 adult *Nephrocerus* specimens was reared, comprising 17 males and 14 females. Jeff Skevington identified 22 dried specimens and 2 in ethanol as *N. atrapilus* and two dried specimens as *N. daeckei*. Five *Nephrocerus* adults in ethanol are in poor condition and not identified. Larvae in the remaining 25 cultures were not successfully reared. Some larvae and puparia were preserved for morphological study and therefore adult flies were not reared or determined. Thirty-one puparial exuviae were preserved in ethanol. Voucher material is deposited at Carnegie Museum of Natural History (CMNH) and the Canadian National Collection of Insects, Arachnids and Nematodes (CNC).

After overwintering, parasitoid larvae were removed form refrigeration and after 43 to 75 days became active, crawling about through the soil medium. This activity lasted about 3 to 8 days, after which the prepupae again became quiescent and soon pupariated. Puparia would initially lack respiratory horns and require several days before the developing pupa would shift inside its puparium and project its respiratory horns, a process similar to that described by Roddy (1955) for the muscid fly *Ophyra aenescens* (Weidemann). Adult pipunculid eclosion occurred 66–101 days (n = 26) from the day of removal from refrigeration, and about ten months after larval emergence from the host (298–321 days, n = 26). There was little difference in developmental time between genders. Adult males eclosed at 66–100 days (n = 13) from refrigeration and females 66–101 days (n = 13).

Crane fly parasitoids were also documented from three other Pennsylvania localities. A single parasitized crane fly was collected at each of these localities. A female *T.* (*Yamatotipula*) *tricolor* Fabricius was collected by light trap at Powdermill Nature Reserve (40°-09′-30″N, 79°-16′-25″W) on 6 August 2002, in Westmoreland County. A female *T.* (*Y.*) *furca* Walker was hand collected at Green's Island, Lake Clarke, along the Susquehanna River (39°-58′-37″N, 76°-28′-05″W) on 7 August 2003, in Lancaster County. A male *T.* (*L.*) *submaculata* Loew was hand collected at Pennsylvania State Game Lands No. 90 (41°-06′-10″N, 78°-28′-15″W) on 26 July 1997, in Clearfield County. A larva emerged from this male host in the field as discussed in the introduction. This record represents the only male crane fly infested with a pipunculid larva. No rearing larva–adult associations were established for these three samples due to the fact that emergent larvae were either preserved or were retrieved from preserved crane fly specimens. They are similar to the larvae from the Boyce Park study site and appear to be *Nephrocerus*.

Results from dissection.—Dissections were performed on 156 ethanol-preserved, adult *Tipula* from Boyce Park. This resulted in our obtaining 25 additional pipunculid larvae from 136 female crane flies but none from 20 male flies. Two distinctive larval forms were observed from these dissections. We believe these belong to two larval instars of one species of parasitoid fly based on observations of several larvae that were preserved shortly before they were ready to molt. These molting larvae still had the penultimate instar's cuticle attached. The posterior spiracles of the ultimate instar are clearly visible under the

partially cast cuticle and its associated posterior spiracles. The posterior spiracles of the two instars are easily distinguishable morphologically and were used in this study to differentiate the larval instars. Due to the uncertainty as to the actual number of instars in the pipunculid larvae, in this paper we do not number the insters, rather use "penultimate" instar for the earlier stage, and "ultimate" for the last instar prior to pupariation.

The dissections yielded 11 ultimate instars and 9 penultimate. Most of the penultimate instars (8) were found with their mandibles facing posteriorly inside the host's abdomen, one was found facing anteriorly. Of the ultimate larvae, 7 were found facing posteriorly, 3 were facing anteriorly and one was unducumented. The penultimate instars were found in crane fly abdominal segments 3 to 7. The ultimate instars were found in abdominal segments 2 to 8. Large, ultimate instars would often occupy two or three consecutive abdominal segments.

In four dissections where parasites were discovered in situ, the crane fly host had at least one of its own mature black eggs (i.e., fully chorionated egg) inside the abdomen. The following details are from these dissections: one host with one mature egg and a penultimate instar parasite; one host with four mature eggs and an ultimate instar parasite; one host with five mature eggs and a penultimate instar; one host with two mature eggs and a penultimate instar parasite. Many dissected non-infested specimens had developing, immature eggs.

There were 56 host crane flies that ultimately had pipunculid prepupae emerge through their body walls. The hosts showed emergence holes through the pleural membrane on either side of the abdomen. Abdominal segments 3–6 typically exhibited an emergence hole, and that hole might also occur between any two consecutive segments. Emergence holes were restricted to the pleural regions and were not found through or between abdominal tergites or sternites. Two hosts with emergence holes contained mature black eggs. One host had only one egg, the other over 50. The majority of the infested hosts had neither black eggs nor any developing immature eggs in the abdominal cavity.

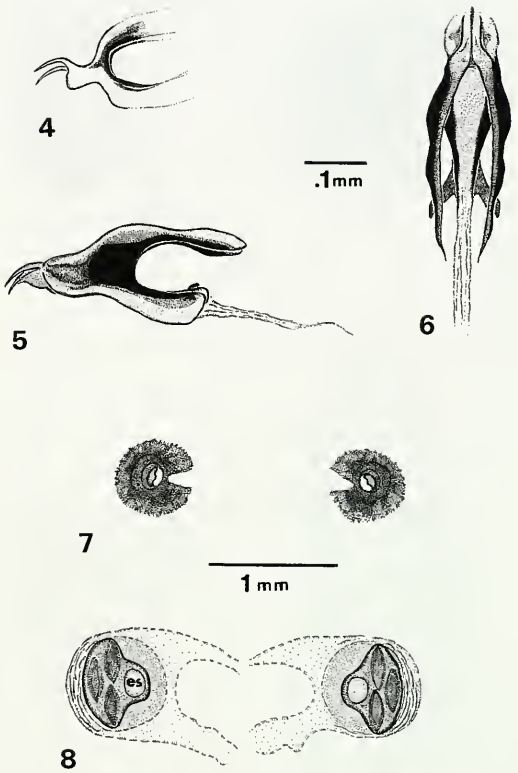
Internal abdominal tissue damage caused by feeding activity of endoparasitic pipunculid larvae was not clearly observed. All infested hosts had reduced fecundity or complete lack of egg production. No infested host was observed to lay eggs, but many unparasitized females deposited mature eggs. Many hosts that contained large, ultimate instar pipunculid larvae had very little fat body or mature eggs in the basal segments; these were, however, present in the more posterior segments 6 to 8 of the same flies. The digestive tract and spermatheca were found to be intact in all hosts. Large, last instar parasitoid larvae may compact internal host tissue into the posterior abdominal segments of the host. One pipunculid larva emerged from a host that contained more than 50 mature eggs. This host was the only one ever observed to have damage to the posterior side of its metathorax. In fact, a single black egg was lodged in the middle of its thoracic cavity.

On one occasion the process of parasitoid emergence from a dead host was observed with the aid of a microscope. The larva placed itself transversely inside the abdomen and pushed its mandibles through the pleural membrane while extending its body against the opposite abdominal wall. The mandibles were not seen to bite or cut through the pleural membrane, but they functioned as a sharp tip to forcefully puncture the membrane. The parasitoid larva emerged from the right pleural region of the host between segments 4 and 5.

Only a single instance of multiple conspecific parasitoids in a single host was observed. An ultimate instar pipunculid larva emerged from one individual of *T. (L.) duplex*. The body of the host crane fly was then dissected and found to contain a penultimate instar *Nephrocerus* larva in segment 6. There was no evidence of multiparasitism (more than one species of parasitoids in the same host) in any host *Tipula* observed in this study.

Summary of results.—This study obtained a total of 81 parasitoid larvae from 309 randomly collected adult crane flies, representing a 26% rate of parasitism. Among these parasitoids, 56 left the hosts voluntarily within 5 days after the capture, and the other 25 were obtained by dissection of the hosts. A high percentage of the parasitoids remaining inside the host were penultimate instar or immature ultimate instars judging from the external morphology of the posterior spiracles. The rate of pipuculid parasitism of female crane flies in this study was 42% (82/193) and the rate was less than 1% (1/119) for host males. Thirty-one of the parasitoids were successfully reared to the adult stage and consisted of two species of Pipunculidae belonging to the genus *Nephrocerus*.

The endoparasitoid pipunculid larvae undergo a rapid active feeding stage inside the adult crane fly hosts for less than 20 days to reach maturity. Mature parasitoids emerged from the hosts in summer; they then go through about ten months (298–321 days) of a non-feeding diapause period before pupariation takes place the following spring.

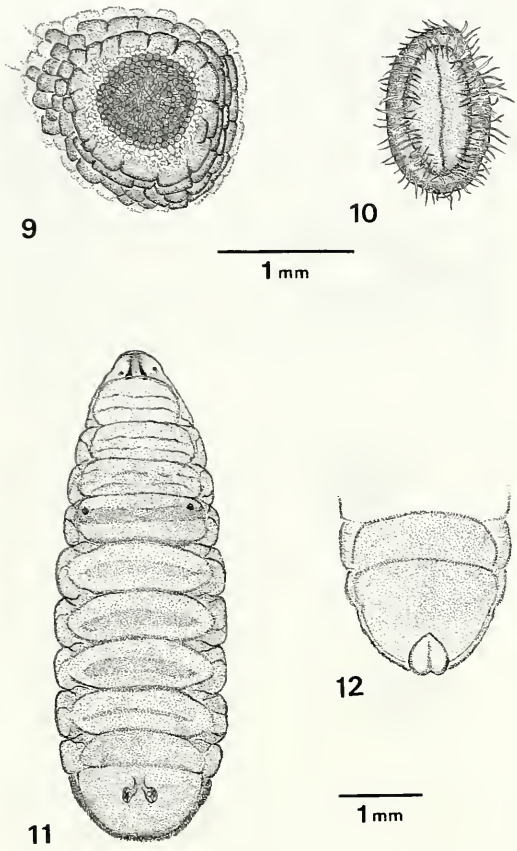



Figs. 4–8. 4–6, Cephalopharyngeal skeleton of *Nephrocerus* larvae. 4, Penultimate instar larva, left lateral view. 5, Ultimate instar larva, left lateral view. 6, Ultimate instar larva, dorsal view. 7–8, Posterior spiracles of *Nephrocerus* larvae. 7, Penultimate instar larva, posterodorsal view. 8, Ultimate instar larva, posterodorsal view.

## Descriptions of Immature Stages of *Nephrocerus*
(Figs. 4–14)

Larva.—Penultimate larval instar. Body length 1.6–3.3 mm; width 0.5–1.1 mm. Body elongate with indistinct segmentation; posterior end without obvious spiracular disc. Cephalopharyngeal skeleton lightly sclerotized with distinct, paired mouth hooks (Fig. 4). Spiracular plates heavily sclerotized, raised mounds with single, round spiracular aperture (Fig. 7).

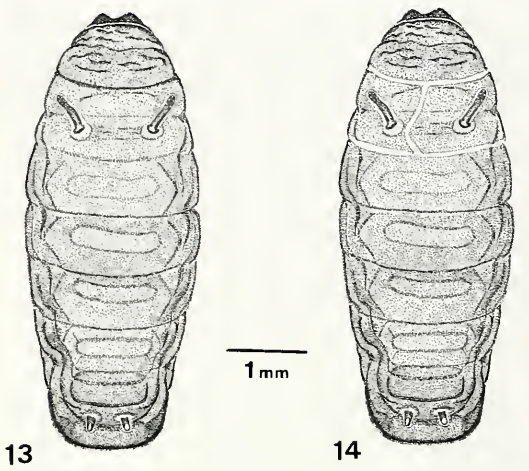
Ultimate larval instar. Body length 4.6–7.8 mm; width 1.9–2.6 mm. White to pale yellow white in color, diapausing larvae honey brown (degree of yellow coloration probably due to amount of fat stored in body). Body oval, stout when contracted, elongated when relaxed (Fig. 11). Acephalic, wrinkled, anterior end tapering to acute tip with mandibles extruded, or truncated with mandibles retracted; posterior half of body broadly




Figs. 9–12. Ultimate larva of *Nephrocerus*. 9, Circular spot on the first abdominal segment, dorsal view. 10, Anal plate on the last abdominal segment, ventral view. 11, Ultimate larva, dorsal view. 12, Inflated anal papillae, ventral view.

rounded. Cuticle of body at 30× magnification with microsculpture of rounded or polygonal "scales", each scale convex in lateral view and varying in diameter depending on location; cuticle covered with microtrichia, especially along ridges of annulations; microtrichia brown, stout and directed backward, or pale and straight.

*Head:* Antenna tiny, bulbous; maxillary palp positioned close to each other along dorsolateral edge of mouth opening, not visible when head is retracted within thorax; cephalopharyngeal skeleton well sclerotized, with paired, sharp, downwardly directed mouth hooks (Figs. 5–6).




Figs. 13–14. Puparium of *Nephrocerus*. 13, Puparium, dorsal view. 14, Puparuim showing line of fracture, dorsal view.

*Thorax:* Anterior spiracle reduced in size and without projection, located on dorsolateral edge of prothorax.

*Abdomen:* Abdomen reduced to six visible segments with secondary annulations forming transverse, elliptical ridges in dorsal or ventral aspect. Pair of darker circular spots (Roddy 1955) approximately 0.14 mm in diameter, dorsolaterally on first visible abdominal segment (Fig. 11) (spots are portals for eversion of respiratory horns in mature puparium); spots with internal microsculpture uniform, grainy, very different from rest of body (Fig. 9). Posterior spiracles without common disc, each elevated on sclerotized plate; each plate with three spiracular openings and one ecdysial scar arranged as in Fig. 8, close to each other on mesal, anterodorsal plane of last visible abdominal segment. Anus ventral on last abdominal segment, with convex longitudinal cleft bordered by defining perianal pad with ring of dense microtrichia (Fig. 10). Anal plate (convex dome) forming anal papillae when inflated (Fig. 12); anal papillae heart shaped when fully inflated with median furrow representing anal slit (larval specimens show anal papillae of varying

degrees of inflation; some larvae observed *in situ* with inflated papillae, all emerged specimens without).

Puparium.—Length 6.5 mm; width 2.6 mm. Body wedge-shaped, broadest just behind respiratory horns on anterior abdomen (Fig. 13); body tapering to truncated mouth opening anteriorly and to evenly rounded posterior end; body heavily sclerotized, reddish brown, with larval segmentation visible to a lesser degree, giving body a slightly bloated appearance. Cuticle of puparium retains scaly appearance, as in larva. Cephalopharyngeal skeleton and cephalic sensory organs retracted within puparium. Pair of respiratory horns on first abdominal segment, appearing within a week of pupariation; horns spikelike, simple, heavily sclerotized, slightly curved, with multiple air holes along distal half of shaft and one single hole near tip.

Note: The respiratory horns of the puparium are located exactly above the circular spots of the larva. The relationship between these two structures has been demonstrated by Roddy (1955). The puparium opens for adult emergence by the lifting of an anterodorsal operculum and the dorsal plate of first abdominal segment. The first abdominal tergum is broken into two pieces along a definite fracture line, each piece with one respiratory horn. The line of fracture is skewed toward the left respiratory horn in anterodorsal view (Fig. 14).

## Discussion

Parasitism of Diptera by other true flies is uncommon. In all known cases the parasites have been identified as species of Tachinidae, and the hosts are larvae of Tipulidae and Tabanidae (Ferrar 1977). Adult Diptera that serve as hosts for other parasitic Diptera are even more rare. Ferrar reported two cases in Australia of tachinid flies attacking adult flies of *Amenia* (Calliphoridae) and *Dasybasis* (Tabanidae). The current study provides some unique observations of host-parasitoid relationships between adult flies of Tipulidae and Pipunculidae.

Adult crane flies are short-lived. In our study area, adult emergence for one species may be spread over one month, and the average individual adult lives about two weeks. This forces parasitic larvae to undergo rapid development. Ferrar (1987) indicated that pipunculids are unusual among Cyclorrhapha in that there are apparently only two larval instars, a condition compatible with the short adult life span of their hosts. There seems to be ample evidence that at least in genus *Chalarus* there are indeed only two larval instars (Jervis 1980). We have noticed only two larval instars for pipunculids in this study but have made no observations on eggs or oviposition and cannot with certainty state that there are in fact just two instars. The penultimate instars show a great range in body size but with identical size and all morphological characteristics of posterior spiracles. The morphology of the ultimate instar is the same as that of the emerged prepupae. These two instars can be easily distinguished by the structure of the posterior spiracles and the cephalopharyngeal skeleton. The posterior spiracles of the penultimate instar have no visible ecdysial scar (Fig. 7) as is present on the ultimate instar (Fig. 8).

Parasitized crane flies do not exhibit altered behavior or deleterious effects with respect to their ability to fly. In the field they all seemed to be healthy and vigorous, indicating that the flight muscles and nervous systems are not disrupted by the parasitoids. This is in contrast with observations of behavior change in parasitism by Pipunculidae in at least some Rice Leafhoppers Yano et al. (1985), and movements impaired of leafhopper hosts due to the damage to the thoracic muscles and nervous system (May 1979). It is likely that the para-

sitoids did not consume any of the vital organs of the hosts. However, developing eggs were absent or noticeably reduced in number in all of the parasitized females, an indication that the reproductive system of the host was consumed by the pipunculid larva. One female host did have about fifty mature eggs left in the posterior end of the abdomen, and in these the chorion was already black. All hosts examined still had intact spermathecae. It therefore seems possible that parasitized hosts might oviposit viable eggs after emergence of the parasitoid. The high percentage 98% (82/83) of hosts being female crane flies may also indicate that feeding on crane fly eggs is required for complete development of the parasitic larvae. The absence or reduction in number of developing eggs within the host's abdominal cavity may also indicate early oviposition by the parasitoid. This high percentage of female crane fly hosts also leads to the speculation that female *Nephrocerus* have the ability to discriminate the sex of adult crane flies, and may strongly favor ovipositing in females.

When the first adult *Nephrocerus* was reared, efforts were made to search for them at the primary study site. Pipunculid species in other genera are considered to have restricted flight times (Hardy 1943). We made 26 collections at various hours of the day between 8:00 am and 9:00 pm, but no adult pipunculids were observed or collected at this site. We did not utilize any specialized techniques for the collection of Pipunculidae, such as Malaise traps, aerial suction traps, attractant sprays on foliage, etc. (Skevington and Marshall 1998). However, a black light collection in May of 2005 in Westmoreland County did yield two female specimens of *N. daeckei*. This may indicate some nocturnal activities of the adult female flies.

Our study demonstrated that adult crane flies in the genus *Tipula* serve as hosts for the parasitoid *Nephrocerus*. The known host species belong to the subgenera *Lunatipula* and *Yamatotipula* within the genus *Tipula*. The majority of species of *Lunatipula* in our study area are univoltine, and they are common spring and summer elements in habitats of mixed deciduous woodlands of oak, hickory, maple, and black cherry. Their larvae are found in the upper soil layers under leaf litter in terrestrial situations. Adult flies of *Yamatotipula* in the study area are bivoltine, first emerging in late April, disappearing in summer, and recurring in August. They are usually found in the vicinity of watercourses in woodland habitats, and their larvae live in a wide variety of aquatic and semi-aquatic habitats. Our rearing results and the black light traps indicated that the primary flight period for *Nephrocerus* is from mid-May to early July, and is most common in mid-June. This record suggests that *Nephrocerus* in our study area is univoltin, which falls within the parameters in the recent study of this genus in North America (Skevington 2005). This observation also concurs with the study of the phenology of three species of *Nephrocerus* occurring in Belgium are univoltine (De Meyer, M., and L. De Bruyn 1989).

The records of *N. atrapilus* reared from *T. (L.) duplex* and *N. daeckei* reared from *T. (L.) mallochi* and *T. (L.) submaculata*, indicate a narrow host-parasitoid relationship and no overlap of hosts between parasitoid species in our study. More research is needed to determine host-parasitoid relationship (i.e. *Nephrocerus* are oligophagous). Other workers have also observed similar occurrences of parasitic larvae emerging from field captured adult crane flies, in Great Britain by Alan Stubbs (J. Skevington, personal communication), in Lithuania by Sigitas Podenas (J. Gelhaus, personal communication), and in Mongolia by J. Gelhaus and S. Podenas

(S. Podenas, personal communication). The documented hosts in North America are five *Tipula* species within the subgenera *Lunatipula* and *Yamatotipula*. In the Old World additional host records have been observed in two subgenera of Tipula *Beringotipula* Savchenko and *Pterelachisus* Rondani, as well as in another genus, *Nephrotoma* Meigen (S. Podenas, personal communication). However, there are no data available for either the sex of these crane flies or the identity of the parasitic larvae. The actual range of host species is still largely unknown. The geographic distribution of the known New World species of *Nephrocerus* is presented by (Skevington 2005: 34) and suggests future survey into additional potential host species. Further investigation may prove that *Nephrocerus* has a much broader range of crane fly hosts.

Host-searching behavior of *Nephrocerus* was not observed in the field. Hardy (1943) described pipunculid females of other genera actively seeking out prey such as leafhoppers. Once the prey is located, the pipunculid will physically grasp the prey and quickly inject an egg into the victim's abdomen or thorax. It is unlikely that *Nephrocerus* females grasp adult crane flies in a similar manner due to the comparatively larger body size of the hosts. Kozanek and Belcari (1997) present some unique character states in morphology of the ovipositor in *Nephrocerus* including well-developed accessory glands and exceptional location of long haired sensilla at the apex of ovipositor. They indicated that the oviposition strategy in these species could be different from other Pipuncuidae.

Based on the facts that female crane flies are the target for the parasitoids, and there is an absence of mature eggs in the host flies, it is suggested that the ovipositing *Nephrocerus* flies might detect pheromonal cues to locate newly eclosed female crane flies. Most species of crane flies mate soon after emergence; the males fly low over the ground in search of females extracting themselves from the pupal exuviae (Pritchard 1983). An alternative host detection scenario may involve female *Nephrocerus* flies detecting male crane fly aggregations near the eclosing females. In both situations the host flies are recently eclosed, teneral and less mobile, therefore more vulnerable to parasitoid oviposition.

In summary, species of the genus *Nephrocerus* represent a pivotal lineage for interpreting the phylogeny of the Pipunculidae. Recent classification studies conducted by Rafael and DeYeyer (1992), and phylogeny analysis based on DNA sequence and morphology by Skevington and Yeates (2000) have both treated *Nephrocerus*, along with genus *Protonephroceurs* as a monophyletic subfamily Nephrocerinae. *Nephrocerus* occupies a transitional position whereby more basal lineages, subfamily Chalarinae, as well as higher lineages, subfamily Pipunculinae, are parasites of auchenorrhynchous Hemiptera (Skevington and Marshall 1998). The knowledge of the larvae and the life histories has always been sought to help unravel these relationships (Skevington 2005). The current study has demonstrated *Nephrocerus* to be endoparasitoids of adult crane flies of the genus *Tipula*. This would make the parasitism of Tipulidae an apomorphic character. The larval biology of *Protonephrocerus*, the sister group of *Nephrocerus* is still unknown.

The mode of infestation in this study is only hypothesized; the specific behavior is still unknown. Further observations on the life history of *Nephrocerus* are needed, especially of host location and oviposition behavior under natural conditions. Of particular value will be observations to determine the time of

oviposition of parasitoids to ensure effective development of parasitoid larvae. An investigation into the possible strategies that the female host species may have evolved to cope with their high pipunculid parasitism rate would be of interest. Since the parasitoids may have a negative impact on the size of the host population, *Nephrocerus* could also be investigated as a potential biological control agent for the introduced European crane fly, *Tipula (Tipula) paludosa* Meigen, and marsh crane fly, *Tipula (Tipula) oleracea* Linnaeus, which have established themselves in the Pacific Northwest and more recently in the eastern United States (Gelhaus 2005, Hoebeke and Krass 2005). Both species are considered to be turf and pasture pests, unlike the forest inhabiting *Tipula* species investigated here.

The likelihood of this parasitism coming to light at this time is probably due to the intensive field collecting of crane flies of both sexes for conducting the faunistic survey. Generally female crane flies tend to be neglected in collecting due to the lack of key characters for specific identification. We believe that there were instances in the past when the pipunculid larvae were observed crawling out of crane fly adults by others and were mistaken for tachinid larvae. Attempts might have also been made to rear the larvae to adult stages but failed due to lack of existing references. Diligent maintenance in an artificial environment of the parasitoid larvae allowed for their survival through the prolonged diapause period that had contributed directly to the unexpected discovery of this unique parasitism. Once we had broken the life history of the parasitoid flies, our subsequent collecting tended to be biased toward visibly infested female crane flies in order to obtain more larvae. This may have led to the appearance of a high infestation rate in our study area.

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## Literature Cited

Alexander, C. P. 1965. The Crane Flies of California. Bulletin of the California Insect Survey 8: 1–269.

Arnaud, P. H., Jr. 1978. A Host-Parasite Catalog of North American Tachinidae (Diptera). U.S.D.A. Miscellaneous Publication No. 1319. 860 pp.

De Meyer, M. 1996. World Catalogue of Pipunculidae (Diptera). Studiedocumenten van het Koninklijk Belgisch Instituut voor Natuurwetenschappen 86: 1–127.

De Meyer, M. and L. De Bruyn. 1989. Seasonal occurrence and voltinism of Pipunculidae (Ddiptera) in Belgium. Bulletin de L'Institut Royal des Sciences Naturelles de Belgique, Entomologie 58: 71–81.

De Meyer, M. and J. H. Skevington. 2000. First addition to the World Catalogue of Pipunculidae. Bulletin de l'Institut Royal des Sciences Naturelle de Belgique, Entomologie 70: 5–11.

Ferrar, P. 1977. Parasitism of other adult Diptera by Tachinidae in Australia. Journal of the Australian Entomological Society 16: 397–401.

———. 1987. Family Pipunculidae, pp. 291–294. *In* A Guide to the Breeding Habits and Immature Stages of Diptera Cyclorrhapha (Part 1:text). Entomonograph vol. 8.

Gelhaus, J. K. 1987. A detritivore *Tipula* (Diptera: Tipulidae) as a secondary host of *Poecilogonalos costalis* (Hymenoptera: Trigonalidae). Entomological News 98(4): 161–162.

———. 2005. The crane fly *Tipula (Tipula) oleracea* (Diptera, Tipulidae) reported from Michigan; a new pest of turfgrass in eastern North America. The Great Lake Entomologist 38: 96–98.

Hardy, D. E. 1943. A revision of Nearctic Dorilaidae (Pipunculidae). University of Kansas Science Bulletin 29(1): 1–231.

———. 1987. Pipunculidae, pp. 745–748. *In* McAlpine, J. F. ed. Manual of Neartic Diptera, Vol. 2. Agriculture Canada Monograph No. 28. 657 pp.

Hoebeke, E. R. and C. Krass. 2005. *Tipula paludosa* Meigen and *T. oleracea* Meigen, European crane flies new to the eastern United States: Potentially serious turfgrass and pasture pest. Proceedings, 16th U.S. Department of Agriculture interagency research forum on gypsy moth and other invasive species 2005: 43.

Jevis, M. A. 1980. Studies on oviposition behaviour and larval development in species of *Chalarus* (Diptera, Pipunculidae), parasites of typhlocybine leafhoppers (Homoptera, Cicadellidae). Journal of Natural History 14: 759–768.

Kozanek, M. and A. Belcari. 1997. Structure of the ovipositor, associated sensilla and spermathecal system of entomoparasitic pipunculid flies (Diptera, Pipunculidae). Journal of Natural History 31: 1273–1288.

May, Y. Y. 1979. The biology of *Cephalops curtifrons* (Diptera: Pipunculidae), an endoparasite of *Stenocranus minutes* (Hemiptera: Delphacidae). Zoological Journal of the Linnean Society 66: 15–29.

Oosterbroek, P. 2005. Catalogue of the Craneflies of the World. The catalogue is on-line available via http://www.science.uva.nl/zma/.

Pritchard, G. 1983. Biology of Tipulidae. Annual Review of Entomology 28: 1–22.

Rennie, J. and C. H. Sutherland. 1920. On the life-history of *Bucentes (Siphona) geniculata*, parasite of *Tipula paludosa* and other species. Parasitology 12: 199–211.

Roddy, L. R. 1955. A morphological study of the respiratory horns associated with the puparia of some Diptera, especially *Ophyra anescnes* (Wied.) Annals of the Entomological Society of America 48: 407–415.

Skevington, J. H. 2005. Revision of Nearctic *Nephrocerus* Zetterstedt (Diptera: Pipunculidae). Zootaxa 977: 1–36.

Skevington, J. H. and S. A. Marshall. 1997. First record of a big-headed fly, *Eudorylas alternatus* (Cresson) (Diptera: Pipunculidae), reared from the subfamily Cicadellinae (Homoptera: Cicadellidae), with an overview of pipunculid-host associations in the Nearctic Region. The Canadian Entomologist 129: 387–398.

———. 1998. Systematics of New World *Pipunculus* (Diptera: Pipunculidae). Thomas Say Publications in Entomology, Monographs, Entomological Society of America, Lanham, Maryland. 201 pp.

Yano., et al. (1985). An evidence for behavioral change in *Nephotettix cincticeps* Uhler (Hemiptera: Deltocephalidae) parasitized by pipunculid flies (Diptera: Pipunculidae). Applied Entomology and Zoology 20(1): 94–96.

Young, C. W. and J. K. Gelhaus. 2000. Crane Flies of Pennsylvania: Preliminary checklist and database development with emphasis on aquatic species. Report submitted to the Wild Resource Conservation Fund, Harrisburg, PA. i–ii, pp. 1–256.